[Frontiers in Bioscience 4, d188-199, February 15, 1999]

# THE ROLE OF INTEGRINS IN THE MALIGNANT PHENOTYPE OF GLIOMAS

**Joon H. Uhm,[1,2] Candece L. Gladson,[3] & Jasti S. Rao[1]**

*Departments of Neurosurgery[1] & Neuro-Oncology,[2] The University of Texas, M.D. Anderson Cancer Center, Houston, Texas, USA 77030, [3] Department of Pathology-Neuropathology, University of Alabama at Birmingham Birmingham, Alabama, USA 35294*

## TABLE OF CONTENTS



## 1. ABSTRACT

Integrins are cell surface receptors that mediate the physical and functional interactions between a cell and its surrounding extracellular matrix (ECM). Expressed as heterodimers, the specific alpha or beta chains that constitute the integrin receptor determine the repertoire of ECM proteins to which a specific integrin may bind (table 1). While classically, the role ascribed to integrins has been that of anchoring cells to the ECM, the more contemporary spectrum of integrin function greatly exceeds that of mere cell adhesion. Recent reports have demonstrated that the interaction between the ECM and cell surface integrins leads to intracellular signaling events that affect cell migration, proliferation, and survival, which in the context of neoplastic cells, can translate directly into the malignant phenotype (1). Indeed, the role of specific integrins in tumorigenesis has been demonstrated in numerous cancer types (table 2).

In primary tumors of the nervous system, the contribution of integrins to the malignant phenotype of gliomas has been an area of significant attention and research in numerous laboratories, including that of ours. As illustrated in table 3, several integrins have been identified as being of key importance in glioma biology. In this article, we review the current knowledge of how these integrins influence the malignant characteristics of gliomas and, as such, how these cell surface receptors may thus represent potential targets in the design of future therapeutics for patients afflicted with gliomas.

## 2. INTEGRINS AND THE INFILTRATIVE BEHAVIOR OF GLIOMAS

The ability of glioma cells to infiltrate brain structures that are adjacent to or distant from the primary tumor site is one of the most important determinants of the poor prognosis associated with these tumors (2). This infiltrative behavior of glioma cells is a function of two phenotypes: migration and invasion. Although the two terms are often used interchangeably in the context of malignant gliomas, there are functional features that distinguish them. Classically, *migration* refers to the capacity of locomotion on the part of tumor cells. In contrast, the process of *invasion* involves migration *plus* the additional feature of a degradative function, typically reflecting the tumor cells' ability to effect degradation of ECM barriers by the liberation of proteolytic enzymes (proteases). For both functions – migration and invasion – there is a direct correlation with tumor grade, with the high grade gliomas demonstrating more extensive migratory and invasive capacities (3). Integrins, by virtue of their cell surface localization and their ability to physically interact with ECM proteins, are ideal candidates to mediate both migration and invasion. In this regard, the process of tumor cell motility/invasion is analogous to the climbing of a ladder, in which the ECM proteins function as the "rungs" of the ladder, while cell surface integrins confer upon the motile cell the ability to physically "grip" these rungs. Moreover, as integrins interact with the cytoskeletal proteins within the cell (figure 1), integrins are well poised to effect alterations in this internal protein-scaffolding

**Table 1.** Pairing of integrin subunits

| Subunit | Pair | Spectrum of ECM protein ligands |
|---|---|---|
| $\alpha_v$ | $\beta_3$ | VN,TS, OP, VWF, TN |
| | $\beta_5$ | VN |
| | $\beta_6$ | FN, TN |
| | $\beta_8$ | ? |
| $\alpha$IIb | $\beta_3$ | VN, Fb |
| $\beta_1$ | $\alpha_1$ | CL, LM |
| | $\alpha_2$ | CL, LM, TN |
| | $\alpha_3$ | CL, LM, FN, ET |
| | $\alpha_4$ | FN, VCAM-1 |
| | $\alpha_5$ | FN |
| | $\alpha_6$ | LM |
| | $\alpha_7$ | ? |
| | $\alpha_8$ | BL |
| | $\alpha_9$ | TN |
| | $\alpha_v$ | FN |

Abbreviations: BL: basal lamina, CL:Collagen, ECM, extracellular matrix, ET,: entactin, Fb: Fibrinogen, FN: Fibronectin, OP: Osteopontin, TS: Thrombospondin, TN: tenascin, VWF: von Willebrand factor

**Table 2.** Integrins associated with cancers of non-CNS origin

| Integrin | Tumor type | References |
|---|---|---|
| $\alpha_2\beta_1$ | Breast | 90,91 |
| $\alpha_3\beta_1$ | Metastases | 92 |
| $\alpha_5\beta_1$ | Ovary | 18,19 |
| | Leukemia | 17 |
| | Osteosarcoma | 93 |
| | Colon | 20 |
| $\alpha_6$ | Head and Neck | 94 |
| | Bladder | 95 |
| | Lung | 96 |
| | Colon | 1 |
| | Melanoma | 97-101 |

**Table 3.** Integrins in glioma biology

| Phenotype | Integrin | Ligand |
|---|---|---|
| Migration/invasion | $\alpha_2\beta_1$ | TN |
| | $\alpha_5\beta_1$ | FN |
| | $\alpha_6\beta_1$ | LM |
| | $\alpha_v\beta_3$ | VN |
| Protease regulation at tumor cell surface | $\alpha_v\beta_3$ | VN |
| Angiogenesis | $\alpha_v\beta_3$ | VN |
| Enhanced survival | $\alpha_v\beta_3$ | VN |

Abbreviations: FN: fibronectin, LM: laminin, TN: tenascin, VN: vitronectin

which is necessary to cause the cell shape changes required for the physical act of cell motility.

### 2.1. Migration on stroma-derived ECM proteins: the role of the beta1 integrin

With the exception of the fibrous meninges that cover the brain surface, virtually all of ECM proteins in the normal adult brain are localized to the perivascular space, where collagen, fibronectin, and laminin constitute the three principal ECM proteins of the blood-brain barrier (4). As this perivascular region (or Virchow-Robin space) is a frequent site of infiltration by glioma cells, the endothelium-derived and pial-glial membrane-derived ECM proteins represent logical candidates to function as potential promoters of glioma migration.

Of the integrin subunits that combine to form the receptors to bind the perivascular ECM (collagen/fibronectin/laminin), the beta-1 chain appears to be a critical component, as it is capable of heterodimerization with numerous different alpha chains and thus underlies the diversity of ligand binding by the beta-1-containing heterodimers (alpha5beta1, fibronectin receptor; alpha6beta1, alpha2beta1, alpha1beta1, laminin receptor; alpha2beta1, alpha3beta1, collagen receptor; see table 1). As discussed below, there is abundant evidence supporting the importance of the beta1 integrin in glioma migration. *In vitro*, neutralizing antibodies directed towards the beta1 integrin lead to a dramatic reduction in glioma migration (5). *In vivo*, the induction of beta1 overexpression in C6 glioma cells that are implanted intracranially into the brain of nude mice leads to diffuse invasion in the brain (6). Given that the beta1 chain is the subunit that is a common denominator among the alpha-beta heterodimers that can bind to the ECM proteins present in the perivascular space (i.e., collagen, fibronectin, laminin), we will review how specific partnerships of the beta1 chain with the various alpha subunits contributes to glioma migration, specifically in relation to laminin and fibronectin.

#### 2.1.1. Beta1 chain as a component of receptors for laminin: alpha6beta1 and others

Of the many ECM proteins that have been evaluated, laminin has been demonstrated to be one of the most permissive substrates for glioma migration *in vitro* (7-10). *In vivo*, evidence also exists to support a pro-migratory role for this protein. Laminin is present in the perivascular region, a location commonly infiltrated by glioma cells *in vivo* (4). In xenograft models, implantation of human glioma cells into the rodent brain was associated with punctate areas of laminin expression, which was presumably host-, rather than tumor-, derived (11). Therefore, the non-neoplastic cells in the brain of endothelial and possibly glial origin may partially provide the ECM substrate that is permissive to the process of glioma infiltration.

The beta1 chain is capable of combining with numerous alpha subunits (alpha 1-9, alphaV); of these alpha-beta heterodimers, many are capable of binding laminin (table 1). Whereas the great majority of these alpha chains (alpha 2-7, alphaV) have been shown to be expressed by glioma cells in tissue culture (9,12), only the alpha2, alpha3 and alpha6 chains have been found to be consistently expressed *in vivo* by human glioma cells (13,14). Of these, alpha3 and alpha6 chains are involved in glioma migration *in vitro*, as neutralizing antibodies directed to either of these alpha subunits leads to dramatic reductions of glioma migration *in vitro* on purified

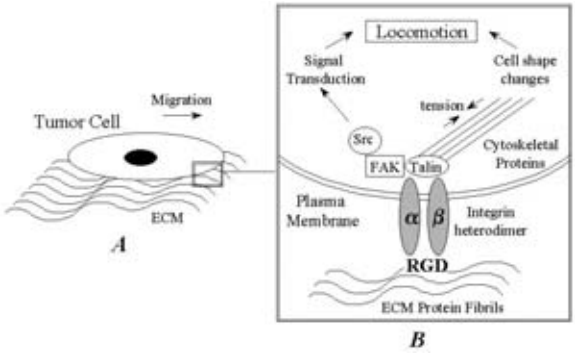


**Figure 1.** Integrins as mediators of cell migration. The process of cell migration involves a dynamic interaction between the cell and its extracellular environment (A). Magnification of the site of interaction between the cell and the proteins of the extracellular matrix (ECM) demonstrates the convergence of numerous proteins to this site (B). Extracellularly, the alpha-beta integrin heterodimer binds non-covalently to the three amino acid sequence, RGD (Arg-Gly-Asp) present in many ECM proteins. Intracellularly, the integrin subunits interact with talin and other proteins of the cytoskeleton, thereby generating the contractile tension and force necessary to induce cell shape changes that accompany cell motility. As well, integrin ligation classically leads to the phosphorylation of focal adhesion kinase (FAK), which in turn, permits the binding of another tyrosine kinase, Src, thereby activating intracellular signal transduction pathways that also contribute to the locomotive process of the migrating cell. (For simplicity, many focal adhesion elements have been omitted from this diagram). Abbreviations: ECM, extracellular matrix; FAK, focal adhesion kinase; RGD, Arginine-Glycine-Aspartate.

laminin. In contrast, antibodies to the alpha2 chain had no discernible effect (5,9).

Interestingly, while glioma migration on laminin is integrin-mediated (i.e., migration was inhibited by neutralizing antibodies to the alpha6 or beta1 subunits that comprise the laminin receptor), cell adhesion to laminin may be a function mediated by both integrins and a non-integrin receptor, such as the 67 kDa laminin receptor (8,9,15). In this regard, neutralizing antibodies directed toward the alpha6 and beta1 subunits inhibit the adhesion of U251MG glioma cells to laminin (15), whereas the anti-adhesive effect of these integrin antibodies on other glioma cell lines is less pronounced. These finding suggests that the processes of glioma migration and adhesion on laminin may be controlled independently, with migration on laminin being integrin-mediated, while the adherence of glioma cells to this ECM substrate being only partially dependent on integrins.

#### 2.1.2. The alpha5beta1 integrin: receptor for fibronectin

The alpha5beta1 integrin is the high affinity receptor for fibronectin, an abundant component of the blood brain barrier. As predicted from the observation that $\beta_1$ facilitates glioma migration, its alpha5 partner seems to promote glioma migration. Fibronectin itself is a permissive substrate for glioma migration (7,9,16). In some studies, neutralizing antibodies to the alpha5 integrin reduce glioma migration *in vitro* (9), suggesting that the beta1 subunit, when heterodimerized with alpha5, may interact with fibronectin in a way that facilitates glioma motility. Conversely, the interaction between integrin receptors and fibronectin may sometimes function as an inhibitor of glioma migration, as neutralizing antibodies to alpha5 have also been associated with enhanced glioma migration (12). Similarly, the overexpression of the alpha5beta1 heterodimer has been associated with a reduction in tumorigenicity in K562 erythroleukemia cells (17), Chinese hamster ovary cells (18,19), and in HT29 colon carcinoma cells (20).

While it remains to be reconciled as to how alpha5beta1 can serve both a facilitatory and inhibitory effect on glioma migration, there are at least two possible explanations. First, the effect may be cell-dependent, as alpha5-neutralizing antibodies demonstrated the differential effects on the migration or invasion of the following glioma cell lines: inhibition of D37MG (9), increased invasion of U138MG cells (12), and no effect on U251.3MG migration (21). Secondly, the effect of alpha5beta1 in glioma migration may depend upon the composition of proteins present in the ECM. In the *in vitro* experiments in which neutralizing antibodies to alpha5 reduced migration (9), glioma cells were seeded onto purified fibronectin. In contrast, in the setting where alpha5-neutralizing antibodies promoted glioma invasion (12), glioma cells were plated onto Matrigel, which contains not only fibronectin, but numerous other ECM proteins in addition to fibronectin, such as entactin, laminin, collagen, and vitronectin, as well as growth factors, thus raising the possibility that other integrins expressed by glioma cells, when ligated by their respective ECM ligand, may influence the effect of the alpha5beta1 integrin on glioma migration.

### 2.2. Integrins that interact with ECM proteins produced by glioma cells

The ECM proteins discussed thus far (fibronectin, laminin, collagen) are derived from the non-neoplastic cell populations in the brain. In contrast to these stroma-derived ECM proteins, several ECM proteins have been shown to be expressed directly by the glioma cells themselves, which include tenascin, vitronectin, hyaluronic acid, osteopontin (22). These glioma-derived ECM proteins may thus function as autocrine factors that promote glioma migration. This section will focus on the integrin-mediated migration on tenascin and vitronectin [migration on hyaluronic acid is CD44-mediated; for literature related to this subject, the reader is referred to a recent review (23) and other reports (14,24-27)].

#### 2.2.1. Tenascin

Tenascin is an adhesive ECM protein whose receptors include multiple integrins, likely reflecting the multi-domain structure of this adhesive protein (28). On endothelial cells, the alpha2beta1 and alphaVbeta3 integrins are capable of binding tenascin, while on

epithelial cells, the alphaVbeta6 and alpha9beta1 function as tenascin receptors (28-31). Of these integrins, the alphaVbeta3 and alphaVbeta6 heterodimers interact with the RGD-site [SRRGDMS in tenascin (28)], one of the peptide domains in ECM proteins recognized by integrins, while alpha2beta1 interacts with tenascin in an RGD-independent manner (31).

Tenascin can be involved in either physiologic or pathologic functions, depending on the circumstances and the cell type in which it is expressed. Outside the nervous system, its physiologic roles include mesenchymal-epithelial interactions during tissue remodeling and differentiation in the gut, kidney, and mammary gland (32-35). In the central nervous system, tenascin promotes cerebellar granule cell migration (36) and neurite outgrowth (37). In terms of cancer biology, tenascin expression has been correlated with the malignant behavior of numerous tumor types (38-40), including carcinomas of the colon (41), breast (42), as well as gliomas (43-46).

With respect to gliomas, the processes of adhesion and migration on tenascin appear to be mediated by distinct integrin heterodimers expressed by glioma cells. The alpha2beta1 integrin is capable of mediating both adhesion and migration of glioma cells on tenascin, a function that appears to be independent of the RGD sequence-containing domain (SRRGDMS) of this ECM protein (30). Tenascin is an extremely permissive substrate for glioma migration, a function attributable to the alpha2beta1 integrin, as neutralizing antibodies to either subunit chain effectively inhibit the migratory capacity (21).

In contrast, the alphaV-containing integrins (alphaVbeta3, alphaVbeta6) that interact with tenascin are capable of mediating adhesion but not migration on this ECM protein. In contrast to the alpha2beta1 integrin, the adhesive function of the alphaV-containing heterodimers is RGD-dependent (28). Moreover, evidence exists to suggest that the alphaV-containing integrins are inhibitory with respect to glioma migration on tenascin, as targeting the alphaV integrin with antisense oligonucleotides (47) or with neutralizing antibodies (48) leads to facilitation of glioma migration on tenascin.

Thus, with respect to glioma biology, tenascin may contain at least two functional domains whose interaction with specific integrin heterodimers can significantly alter the migratory behavior of glioma cells. The RGD-independent domain of tenascin facilitates both adhesion and migration through its interaction with the alpha2beta1 integrin. Alternatively, while the RGD-containing domain, through its binding to the alphaV-containing integrins, facilitates cell adhesion, this interaction may actually inhibit cell migration on tenascin. Thus, the degree of glioma migration on tenascin may depend on the net balance between two potentially opposing forces: the pro-migratory function of the alpha2beta1 integrin versus the anti-migratory tendency of the alphaV-containing integrins.

*In vivo*, while tenascin is expressed during the development of a various organs, including the brain (see above), the normal adult brain is devoid of tenascin expression. However, under neoplastic conditions, tenascin expression becomes evident, with its levels correlating with the grade of the glioma (43). The expression of tenascin is restricted to the tumor mass, with the adjacent normal brain remaining negative for tenascin. In this regard, two cell populations within the tumor bulk synthesize tenascin: the glioma cells and endothelial cells.

While there is abundant and direct evidence in the *in vitro* literature supporting a role for tenascin in glioma migration, the evidence to generalize this role to the *in vivo* arena is more circumstantial. For example, both tenascin expression and glioma migration/invasion *in vivo* increase in parallel with tumor grade. As well, tenascin expression by glioma cells *in vivo* has also been correlated with the occurrence of leptomeningeal spread of glioma cells in patients (49). In these circumstances, it is possible that, similar to the *in vitro* condition, tenascin (whether derived from glioma cells or endothelium) interacts with cell surface integrins of gliomas to facilitate the migration process.

The expression of tenascin by endothelial cells is much more prominent than that by glioma cells *in vivo* (50,51). Both immunohistochemical and *in situ* hybridization analyses which demonstrate intense labeling of the vasculature (especially hyperplastic vessels) and the juxtaposed basement membrane , thereby suggesting an angiogenic role for tenascin through its interaction with endothelial alpha2beta1 and alphaVbeta3 integrins. Thus, the correlation between tenascin expression and glioma grade may be a reflection of the degree of angiogenesis, which also increases with tumor grade. In support of a predominantly angiogenic, rather than invasive, role for tenascin, the expression of this ECM protein is also abundant in the hyperproliferative vessels of juvenile pilocytic astrocytomas (JPAs), which are characteristically highly angiogenic, but non-invasive tumors (43).

Regardless of its predominant function *in vivo* – migratory versus angiogenic – the restricted expression of tenascin to the tumor mass has been exploited by Bigner's group, in which tenascin serves as a target for directing anti-tumor therapeutics selectively to the tumor bed (see section 5.2).

### 2.2.2. Vitronectin

Vitronectin (VN) is a multifunctional 75 kDa glycoprotein that constitutes the major adhesive component of serum, and permits the attachment and spreading of cells propagated in serum-containing media in culture (52). *In vivo*, the physiological roles of VN have been shown to include the regulation of thrombosis, fibrinolysis, and complement-mediated cell lysis in the circulation. In the central nervous system, VN expression is abundant in the developing retina, where it functions as a positive modulator of neurite outgrowth (53) and contributes to other functions in the developing brain (54).

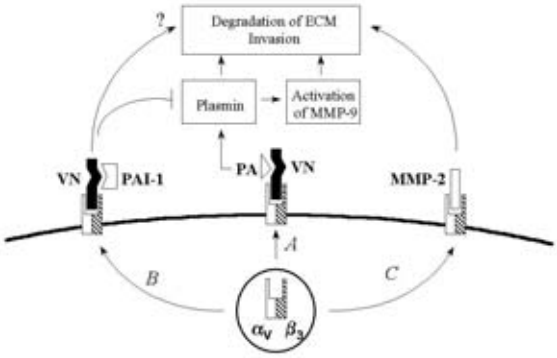


**Figure 2.** Vitronectin and its receptor: regulation of protease activity. The alphaVbeta3 integrin heterodimer comprises the classic receptor for vitronectin (VN). Depending on its conformational state, VN can bind either (A) the serine protease plasminogen (PA), or (B) the protease inhibitor, PAI-1 (plasminogen activator inhibitor type 1). When plasminogen is bound to the VN-alphaVbeta3 complex, plasminogen is converted to its active form, plasmin. Plasmin, in turn, contributes to glioma cell invasion by directly degrading ECM proteins and/or by the proteolytic activation of metalloproteinases, such as MMP-9, from its pro-enzyme form to the enzymatically active form. When the VN-alphaVbeta3 complex binds PAI-1 (B), the conversion of plasminogen to plasmin is impeded. While this should presumably decrease tumorigenicity by way of inhibiting the invasive process, PAI-1 overexpression has been paradoxically associated with increased tumorigenicity in numerous tumors, including gliomas. The presence of PAI-1 at the cell surface may, in part, function to stabilize cell-ECM interactions, which may also be necessary for the process of cell motility (see Section 2.3 for discussion). Recently, the alphaVbeta3 heterodimer has been shown to also function as a receptor for another metalloproteinase, MMP-2, which in turn, can effect ECM protein degradation to contribute to the invasive phenotype. Abbreviations: VN, vitronectin; MMP-9, matrix metalloproteinase-9; PA, plasminogen; PAI-1, plasminogen activator inhibitor type 1.

Although the normal adult cortex and white matter are devoid of VN, VN is re-expressed in the brain parenchyma by malignant gliomas. In particular, VN is expressed by glioma cells themselves, as demonstrated by immunohistochemical (55) and *in situ* hybridization analyses (56). Moreover, several features of VN expression by glioma cells *in vivo* support a potential role for this ECM protein in the malignant phenotype of gliomas (55,56). 1) VN expression by glioma cells *in vivo* correlates with tumor grade, being most abundant in gliomas of high grade histology (glioblastoma; highly invasive), less in intermediate-grade gliomas, and virtually absent in low-grade lesions. 2) The expression of VN is largely restricted to the tumor cells at the tumor-brain interface, the site at which the process of migration/invasion is most active. 3) *In vivo*, glioma cells also express the two cognate receptors for VN, the alphaVbeta3 and alphaVbeta5 integrins. In particular, the alphaVbeta3 integrin complex is expressed specifically by glioma cells at the advancing tumor margin, the area in which VN expression is especially abundant. Taken together, the colocalization of an adhesive ECM protein as well as its receptor (alphaVbeta3 integrin) to the advancing margin of the tumor strongly suggests that VN and its integrin receptor participate in the process of tumor migration and invasion.

*In vitro*, the evidence supporting a role for VN and its integrin receptors in glioma migration is more direct. While VN is more permissive than collagen for U-251MG migration using a haptotactic double chamber Boyden type migration assay, it is not as permissive as collagen or laminin if measured in a radial monolayer migration assay (57). Nonetheless VN does support both adhesion and migration of glioma cells (55-57). As well, the treatment of glioma cells with neutralizing antibodies to the alpha (alphaV) and beta (beta3) subunits of the classic VN receptor leads to a dramatic reduction in glioma migration (12). Conversely, when glioma cells are treated with platelet-derived growth factor (PDGF), which is a potent stimulator of glioma motility, the consequent increase in glioma migration is due in part to integrin alphaVbeta3 (58). Cell motility requires that the cell alternates between detachment from extracellular matrix and its reattachment to the matrix. In this regard, the internalization of vitronectin by the alphaVbeta3 integrin permits the disassembly of focal adhesions that is necessary for the detachment process, while the subsequent redistribution of the alphaVbeta3 integrin heterodimer to the cell surface induced by PDGF re-establishes the cell's physical contact with the matrix (58). Thus, the ability of alphaVbeta3 and alphaVbeta5 integrins to promote glioma motility may be related not only to their ability adhere to the extracellular matrix, but as well by their ability to detach from the matrix by internalizing their ligand, vitronectin (15).

### 2.3. Integrins in the regulation of protease activity: focus on the alphaVbeta3 integrin

The interaction between the alphaVbeta3 integrin and vitronectin provides the glioma cell with not only the physical substrates required for motility, but may also contributes to the process of tumor migration by alternate mechanisms as well. Specifically, the alphaVbeta3 integrin may influence tumor migration and invasion by regulating the localization and activation of tumor-derived proteases at the plasma membrane of the tumor cell.

As illustrated in figure 2, the alphaVbeta3 integrin typically binds its classic ligand, vitronectin. In turn, vitronectin, depending upon its conformation, is capable of binding either a protease (plasminogen) or a protease inhibitor (PAI-1; plasminogen activator inhibitor type 1) (59). This unique ability of alphaVbeta3 to form a trimolecular complex that can either promote or inhibit serine protease activity may permit this integrin to regulate the net proteolytic activity at the tumor cell surface. When the trimolecular complex binds plasminogen and therefore favors proteolysis, local degradation of protein- and cellular barriers may ensue to facilitate invasion, thereby clearing a path over which tumor cells migrate or invade.

Conversely, the binding of the protease inhibitor (PAI-1) may lead to the stabilization of integrin-ECM interactions that provide the necessary "traction" or "grip" necessary for tumor cell locomotion (59). In this regard, the overexpression of the protease inhibitor, PAI-1, has paradoxically been correlated with increased tumorigenicity of various peripheral tumors (60-66) as well as gliomas (67-70). As such, the classic anti-invasive role ascribed to protease inhibitors may be somewhat restrictive, as protease inhibitors, such as PAI-1, may also contribute to the migratory/invasive phenotype.

In addition to its ability to regulate the activity of the serine proteases, such as the plasminogen/plasmin system, the alphaVbeta3 integrin also impacts upon the activity of matrix-metalloproteinases (MMPs) at the tumor cell surface by two mechanisms. First, the plasminogen that is bound to the alphaVbeta3-vitronectin complex at the cell surface is ultimately converted to its active form, plasmin, which is the principal enzyme that proteolytically converts the majority of pro-MMPs to their active counterparts by cleavage of the leader peptide (71-73). Secondly, the alphaVbeta3 integrin can also directly bind activated MMP-2, thereby concentrating this protease to the tumor cell surface (74). Hence, alphaVbeta3 is a multi-functional integrin; it serves as a physical link between the tumor cell and ECM ligands such as vitronectin for cell locomotion, as well as providing the tumor cell with the ability to concentrate and regulate protease function to the cell surface, thereby regulating the infiltrative capacity of glioma cells. Moreover, this integrin is also involved in the process of angiogenesis and may also contribute to the chemoresistant phenotype of gliomas, which is discussed below (see section 4).

## 3. INTEGRINS IN GLIOMA PROLIFERATION

The interaction between the cell surface integrin and ECM may also impact upon cell proliferation. One of the postulates that describes the relationship between cell proliferation and its interaction with the ECM is that of the "go or grow" hypothesis. Central to this postulate is that cell migration ("go") and proliferation ("grow") theoretically cannot occur simultaneously, presumably as the internal cytoskeleton of the cell cannot support both phenomena concurrently. Support for this premise was originally observed in fibroblasts, in which the ligation of the fibronectin receptor (alpha5beta1 integrin) is associated with exit from the cell cycle (1). Similarly, the ligation of the alpha5beta1 integrin on colonic carcinoma cells leads to a reduction in proliferation rate (20). In gliomas, Giese *et al*. have demonstrated similar findings (75). Using an *in vitro* migration assay, in which the radial migration rate away from a seeded pellet of tumor cells is quantified, they were able demonstrate a gradient in cell proliferation/cycling. In this regard, the proportion of cells in cycle (as assessed by BrdU labeling) was much higher for non-migratory cells near the core compared to the low BrdU labeling index for highly migratory cells at the periphery. Thus, while stationary cells residing in the tumor core in the model by Giese *et al*. are highly proliferative, the cells at the periphery, while less proliferative, take on a more migratory and thus infiltrative phenotype. As such, the glioma cell may essentially trade one malignant phenotype of proliferation for the other of migration/infiltration. Whether this observation could be extrapolated to other cell types or to the *in vivo* setting is less clear. In the context of brain tumor cells *in vivo*, approximately 25-30% of the tumor cell population is in cycle. Of the remaining ~70% of the tumor, a proportion of these constitutes the pool of cells that are capable of migration and infiltration into the surrounding brain. As such, future therapeutics for brain tumors will likely need to target the processes of cell proliferation simultaneously with that of cell migration.

## 4. THE ALPHAV AND BETA3/5 INTEGRINS AS MEDIATORS OF CELL SURVIVAL: ROLE IN ANGIOGENESIS & CHEMORESISTANCE

While the receptors for vitronectin, the alphaVbeta3 and alphaVbeta5 integrins, are expressed by glioma cells and clearly contribute to their pathogenesis, the role of vitronectin receptors in the biology of endothelial cells may also contribute indirectly to glioma tumorigenesis. In particular, Cheresh's group has pioneered the concept that the alphaVbeta3 integrin expressed by endothelial cells plays a critical role in the process of angiogenesis, as ligation of the alphaVbeta3 integrin is associated with enhanced survival of the endothelial cell both *in vitro* and *in vivo*. Ligation of the endothelial alphaVbeta3 integrin results in intracellular signals that leads to the increased expression of the antiapoptotic protein, Bcl-2, with a coordinate reduction in the levels of the proapoptotic protein, Bax (76). This stoichiometric change consequently increases the Bcl-2:Bax ratio, thereby tipping the intracellular balance in favor of cell survival through the inhibition of apoptosis. Conversely, while activation of the endothelial alphaVbeta3 integrin enhances cell survival, Cheresh's group has also shown that disruption of this receptor with neutralizing monoclonal antibody, LM609, effectively inhibits angiogenesis *in vivo* as a result of the induction of massive apoptosis of endothelial cells (77). While the anti-angiogenic effects of alphaVbeta3 antagonism, either via neutralizing antibodies (77,78) or recently developed peptidomimetic inhibitors (79,80), have been demonstrated in peripheral tumors, the potential utility of targeting the alphaVbeta3 of the endothelial cells in primary brain tumors remains to be assessed. This may represent a useful target, as mRNA protein levels for alphaVbeta3 integrin have been shown to be upregulated on the endothelial cells of small capillaries in malignant gliomas (81).

Given that the alphaVbeta3 integrin (as well as alphaVbeta5, the other receptor for VN) is also expressed on the surface of malignant glioma cells *in vivo* (55,56), we have recently pursued the hypothesis that these VN receptors may also confer a survival advantage to the glioma cells (unpublished results). In this regard, we have shown that ligation of either receptor for VN – alphaVbbeta3 or alphaVbeta5 – is associated with enhanced survival of glioblastoma cells. Moreover, the enhanced cell survival conferred by these two VN receptors

translates into the phenotype of chemoresistance, a feature that typifies high grade gliomas. Similar to the observation in endothelial cells (76), activation of VN receptors in glioma cells was associated with the increased expression of anti-apoptotic proteins – Bcl-2 as well as Bcl-$X_L$. As levels of the proapoptotic protein, Bax, did not change, the net effect was to tip the balance in favor of cell survival by way of inhibiting apoptosis. As such, the interaction between the vitronectin receptors and vitronectin may contribute to the phenotype of treatment resistance in malignant gliomas via an autocrine loop, as glioma cells express VN as well as its cognate receptors.

## 5. CONCLUSIONS AND PERSPECTIVES

Although the role traditionally ascribed to the interaction between cell surface integrins and the proteins of the extracellular matrix was one of simple cell adhesion, research over the past decade have demonstrated the importance of the cell-ECM interaction in a diverse set of functions. In the context of malignant gliomas, integrins impact upon virtually all features that comprise the "malignant phenotype," encompassing migration, invasion, angiogenesis, and resistance to anti-tumor therapeutics. Given the diversity of functions of integrins and ECM proteins in glioma tumorigenesis, the integrin-ECM interaction thus constitutes a logical area for further research as well as a potential target for therapeutic strategies.

### 5.1. Integrins: what is their role in the low- and intermediate-grade gliomas?

Gliomas can be categorized by a three-grade system, consisting of the low-, intermediate-, and high-grade (glioblastoma multiforme) histologies. As the great majority of investigations in glioma research are focused on the high grade tumors, the contribution of integrins and ECM proteins to the neoplastic properties of these tumors has been well characterized. However, the role of integrins and ECM proteins in the biology of the lower grade tumors remains relatively unaddressed.

While the low- and intermediate-grade histologies are by definition less aggressive than the high grade glioblastomas, these less advanced gliomas can also manifest the menacing features that stem from their migratory capacity. As noted in Section 2, the functional distinction between migration and invasion is that while *migration* simply refers to cell motility or locomotion, the process of *invasion* involves migration *plus* the cell's ability to degrade barriers by way of proteases. In contrast to high grade/glioblastoma tumors, the lower grade gliomas are typically less *invasive*, as they usually express significantly less proteases necessary for degrading the surrounding protein- and cellular barriers. However, many of these tumors are still capable of motility, and are thus able to infiltrate the normal brain.

The prototypical clinical situation in which the migratory capacity of a low-grade glioma is the major determinant of poor patient prognosis is that of gliomatosis cerebri. In this condition, the histological analysis of the biopsy or resected sample often reveals the hallmarks of a "low-grade" glioma, with little or no evidence for proliferation, pleomorphism, or vascular proliferation. However, despite their low-grade microscopic features, these tumors are, by definition, highly infiltrative, with tumor involving not only multiple contiguous lobes in one hemisphere, but often crossing via white matter tracts to the contralateral hemisphere. In this context, we are in need of delineating the integrins as well as other factors contributing the migratory behvavior of these cells, as this condition clearly exemplifies how migration alone can contribute to poor patient outcome.

### 5.2. Targeting specific integrins and ECM proteins

Of the numerous integrin chains expressed by glioma cells *in vivo*, the beta1 subunit may be one of the most important components in the process of tumor migration and invasion, given its ability to heterodimerize with various alpha subunits to form the receptors for the ECM proteins permissive for glioma migration (see section 2.1). For example, the partnership of $\beta_1$ with alpha subunits 2,3,5, and 6 constitute the receptors for laminin, fibronectin, and collagen, which comprise the major ECM proteins of the perivascular basement membrane, a region often infiltrated by glioma cells. The alphaV integrin, when paired with the beta3 chain, forms the vitronectin receptor, which mediates not only migration, but also functions to concentrate both the serine- and metallo-proteinases to the plasma membrane of the glioma cell (**figure 2**), thereby further contributing to the migratory and invasive phenotype. Collectively, these integrin subunits constitute the pro-migratory integrins and thus would pose logical targets for inhibition.

Integrins may potentially be targeted through numerous approaches. At one level, a specific pro-migratory alpha or beta subunit can be targeted using antisense oligonucleotide methods (47). Conversely, it is conceivable that an anti-migratory integrin can be targeted for overexpression using gene therapy approaches. At the next level, the alpha-beta heterodimer complex can be inhibited in several ways. As some integrin heterodimers interact with the RGD-amino acid sequence of the ECM macromolecule (figure 1), this interaction is amenable to potential pharmacologic inhibition by the use of soluble RGD-containing peptides (82,83). However, the RGD sequence is present in many ECM proteins; moreover, many different alpha-beta heterodimers are capable of binding to the RGD domain. Consequently, standard soluble linear peptides would be not be able to offer selective inhibition of a specific heterodimer. Rather, in contrast to linear RGD peptides, cyclic RGD-containing peptides are more selective in their ability to antagonize specific integrin heterodimers, namely the receptors for vitronectin (alphaVbeta3 and alphaVbeta5 integrins). As well, a neutralizing monoclonal antibody specifically directed against a target heterodimer would offer selectivity; indeed, Cheresh's group has used the LM609 antibody to target specifically the alphaVbeta3 complex both *in vitro* as well as *in vivo* (76-78). More recently, peptidomimetic inhibitors selective for the alphaVbeta3 heterodimer have also demonstrated anti-tumor effects *in*

*vivo*, in which this inhibitor was able to inhibit the growth of germ cell tumors in a nude mouse model (79,80).

In addition to targeting the integrin chains or heterodimers, the ECM proteins that interact with these integrins can also be targeted for potential therapeutic design. For gliomas, this concept has been pursued by Bigner and others, who have identified the ECM protein, tenascin, as a target for immune-directed therapy. Taking advantage of the observation that the *in vivo* expression of tenascin in the adult brain is restricted to primarily the blood invessels in the glioma mass (see section 2.2.1), this group has developed a tenascin-specific antibody to which a radioisotope ($I^{131}$) has been conjugated, thereby selectively targeting the tumor mass for immune-directed radiation delivery (84-86). Based upon encouraging results in the nude mouse model (87) and initial patient evaluations (88), they have recently completed a phase I study of this agent in glioma patients (89). Given that another ECM protein, vitronectin, is also selectively expressed by the glioma tumor mass, vitronectin may also be amenable to a similar therapeutic approach.

Thus far, while the role of integrins and extracellular matrix proteins in glioma tumorigenesis has been well substantiated, integrins have not yet been targeted for therapeutics. Of the integrins expressed by gliomas, the alphaVbeta3 may be one of the most logical candidates for antagonism, as its expression of this heterodimer by glioma cells contributes to the processes of glioma migration, protease activation (and thus invasion), and possibly chemoresistance. Moreover, as the alphaVbeta3 integrin is also highly expressed by the hyperplastic capillaries in these tumors, antagonism of this integrin may also impact upon angiogenesis. Thus, given its pleiotropic impact on glioma biology, targeting this integrin may impact upon multiple facets of the malignant phenotype. While antibodies and recently developed small molecule inhibitors directed towards alphaVbeta3 have shown promising results in peripheral tumors, such as melanoma and germ cell tumors, their potential efficacy in the context of malignant gliomas remains to be addressed; these are studies that are currently being assessed in our laboratory.

## 5. ACKNOWLEDGMENTS

JHU is a recipient of the Clinician-Scientist Award from the Medical Research Council (MRC) of Canada. This work was supported in part by CA59958 and NS34856 from the NCI and the NINDS (CLG) and CA56792, CA75557 and CA76350 from the NCI (JSR).

## 6. REFERENCES

1. Varner, J.A. and Cheresh, D.A. Integrins and cancer. *Curr Opin Cell Biol* 8, 724-730 (1996)

2. Berens, M.E., Rutka, J.T., Rosenblum, M.L. Brain tumor epidemiology, growth, and invasion. *Neurosurg Clin N Am* 1, 1-18 (1990)

3. Friedlander, D.R., Zagzag, D., Shiff, B., Cohen, H., Allen, J.C., Kelly, P.J., Grumet, M. Migration of brain tumor cells on extracellular matrix proteins in vitro correlates with tumor type and grade and involves alphaV and beta1 integrins. *Cancer Res* 56, 1939-1947 (1996)

4. Rutka, J.T., Apodaca, G., Stern, R., Rosenblum, M. The extracellular matrix of the central and peripheral nervous systems: structure and function. *J Neurosurg* 69, 155-170 (1988)

5. Tysnes, B.B., Larsen, L.F., Ness, G.O., Mahesparan, R., Edvardsen, K, Garcia-Cabrera, I., Bjerkvig, R. Stimulation of glioma-cell migration by laminin and inhibition by anti-alpha3 and anti-beta1 integrin antibodies. *Int J Cancer* 67, 777-784 (1996)

6. Paulus, W., Baur, I., Beutler, A.S., Reeves, S.A. Diffuse brain invasion of glioma cells requires beta1 integrins. *Lab Invest* 75, 819-826 (1996)

7. Giese, A., Rief, M.D., Loo, M.A., Berens, M.E. Determinants of human astrocytoma migration. *Cancer Res* 54, 3897-3904 (1994)

8. Giese, A., Rief, M.D., Tran, N.L., Berens, M.E. Specific attachment and migration of human astrocytoma cells on human but not murine laminin. *Glia* 13, 64-74 (1995)

9. Haugland, H.K., Tysnes, B.B., Tysnes, O. Adhesion and migration of human glioma cells are differently dependent on extracellular matrix molecules. *Anticancer Res* 17, 1035-1043 (1998)

10. Koochekpour, S., Merzak, A., Pilkington, G.J. Extracellular matrix proteins inhibit proliferation, upregulate migration and induce morphological changes in human glioma cell lines. *Eur J Cancer* 31A, 375-380 (1995)

11. Pedersen, P.H., Marienhagen, K., Mrk, S., Bjerkvig, R. Migratory pattern of fetal rat brain cells and human glioma cells in the adult rat brain. *Cancer Res* 53, 5158-5165 (1993)

12. Paulus, W. and Tonn, J. Basement membrane invasion of glioma cells mediated by integrin receptors. *J Neurosurg* 80, 515-519 (1994)

13. Paulus, W., Baur, I., Schuppan, D., Roggendorf, W. Characterization of integrin receptors in normal and neoplastic human brain. *Am J Pathol* 143, 154-163 (1993)

14. Gingras, M.C., Roussel, E., Bruner, J.M., Branch, C.D., Moser, R.P. Comparison of cell adhesion molecule expression between glioblastoma multiforme and autologous normal brain tissue. *J Neuroimmunol* 57, 143-153 (1995)

15. Pijuan-Thompson, V. and Gladson, C.L. Ligation of integrin alpha5beta1 is required for internalization of vitronectin by integrin alphavbeta3. *J Biol Chem* 272, 2736-2743 (1997)

16. Goldbrunner, R.H., Haugland, H.K., Klein, C.E., Kerkau, S., Roosen, K, Tonn, J.C. ECM dependent and

integrin mediated tumor cell migration of human glioma and melanoma cell lines under serum-free conditions. *Anticancer Res* 16, 3679-3687 (1996)

17. Symington, B.E. Fibronectin receptor overexpression and loss of transformed phenotype in a stable variant of the K562 cell line. *Cell Reg* 1, 637-648 (1990)

18. Schreiner, C., Fisher, M., Hussein, S., Juliano, R.L. Increased tumorigenicity of fibronectin receptor deficient Chinese hamster ovary cell variants. *Cancer Res* 51, 1738-1740 (1991)

19. Giancotti, F.G. and Ruoslahti, E. Elevated levels of the alpha5beta1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* 60, 849-859 (1990)

20. Varner, J.A., Emerson, D.A., Juliano, R.L. Integrin alpha 5 beta 1 expression negatively regulates cell growth: reversal by attachment to fibronectin. *Mol Biol Cell* 6, 725-740 (1995)

21. Deryugina, E.I. and Bourdon, M.A. Tenascin mediates human glioma cell migration and modulates cell migration on fibronectin. *J Cell Sci* 109, 643-652 (1996)

22. Saitoh, Y., Kuratsu, J., Takeshima, H., Yamamoto, S., Ushio, Y. Expression of osteopontin in human glioma. Its correlation with the malignancy. *Lab Invest* 72, 55-63 (1995)

23. Baltuch, G.H., de Tribolet, N., Van Meir, E.G. Expression of the CD44 adhesion molecule in tumours of the central and peripheral nervous system. *J Neurooncol* 26, 191-198 (1995)

24. Okada, H., Yoshida, J., Sokabe, M., Wakabayashi, T., Hagiwara, M. Suppression of CD44 expression decreases migration and invasion of human glioma cells. *Int J Cancer* 66, 255-260 (1996)

25. Ge, L., Resnick, N.M., Ernst, L.K., Salvucci, L.A., Asman, D.C., Cooper, D.L. Gene therapeutic approaches to primary and metastatic brain tumors: II. ribozyme-mediated suppression of CD44 expression. *J Neurooncol* 26, 251-257 (1995)

26. Kaaijk, P., Troost, D., Morsink, F., Keehnen, R.M., Leenstra, S., Bosch, D.A., Pals, ST. Expression of CD44 splice variants in human primary brain tumors. *J Neurooncol* 26, 185-190 (1995)

27. Koochekpour, S., Pilkington, G.J., Merzak, A. Hyaluronic acid/CD44H interaction induces cell detachment and stimulates migration and invasion of human glioma cells in vitro. *Int J Cancer* 63, 450-454 (1995)

28. Prieto, A.L., Edelman, G.M., Crossin, K.L. Multiple integrins mediate cell attachment to cytotactin/tenascin. *Proc Natl Acad Sci USA* 90, 10154-10158 (1993)

29. Bourdon, M.A. and Ruoslahti, E. Tenascin mediates cell attachment through an RGD-dependent receptor. *J Cell Biol* 108, 1149-1155 (1989)

30. Sriramarao, P., Mendler, M., Bourdon, M.A. Endothelial cell attachment and spreading on human tenascin is mediated by alpha 2 beta 1 and alpha v beta 3 integrins. *J Cell Sci* 105, 1001-1012 (1993)

31. Yokosaki, Y., Palmer, E.L., Prieto, A.L., Crossin, K.L., Bourdon, MA, Pytela, R., Sheppard, D. The integrin alpha 9 beta 1 mediates cell attachment to a non-RGD site in the third fibronectin type III repeat of tenascin. *J Biol Chem* 269, 26691-26696 (1994)

32. Erickson, H.P. and Bourdon, M.A. Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. *Annu Rev Cell Biol* 5, 71-92 (1989)

33. Chiquet-Ehrismann, R., Mackie, E.J., Pearson, CA, Sakakura, T. Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell* 47, 131-139 (1986)

34. Derr, L.B., Chiquet-Ehrismann, R., Gandour-Edwards, R., Spence, J., Tucker, R.P. The expression of tenascin-C with the AD1 variable repeat in embryonic tissues, cell lines and tumors in various vertebrate species. *Differentiation* 62, 71-82 (1997)

35. Mackie, E.J., Chiquet-Ehrismann, R., Pearson, CA, Inaguma, Y., Taya, K., Kawarada, Y., Sakakura, T. Tenascin is a stromal marker for epithelial malignancy in the mammary gland. *Proc Natl Acad Sci USA* 84, 4621-4625 (1987)

36. Husmann, K., Faissner, A., Schachner, M. Tenascin promotes cerebellar granule cell migration and neurite outgrowth by different domains in the fibronectin type III repeats. *J Cell Biol* 116, 1475-1486 (1992)

37. Lochter, A., Vaughan, L., Kaplony, A., Prochiantz, A., Schachner, M, Faissner, A. J1/tenascin in substrate-bound and soluble form displays contrary effects on neurite outgrowth. *J Cell Biol* 113, 1159-1171 (1991)

38. Sakai, T., Furukawa, Y., Chiquet-Ehrismann, R., Nakamura, M., Kitagawa, S, Ikemura, T., Matsumoto, K. Tenascin-X expression in tumor cells and fibroblasts: glucocorticoids as negative regulators in fibroblasts. *J Cell Sci* 109, 2069-2077 (1996)

39. Schenk, S., Muser, J., Vollmer, G., Chiquet-Ehrismann, R. Tenascin-C in serum: a questionable tumor marker. *Int J Cancer* 61, 443-449 (1995)

40. Chiquet-Ehrismann, R. Tenascin and other adhesion-modulating proteins in cancer. *Sem Cancer Biol* 4, 301-310 (1993)

41. Sakai, T., Kawakatsu, H., Hirota, N., Yokoyama, T., Sakakura, T., Saito, M. Specific expression of tenascin in

human colonic neoplasms. *Br J Cancer* 67, 1058-1064 (1993)

42. Inaguma, Y., Kusakabe, M., Mackie, E.J., Pearson, C.A., Chiquet-Ehrismann, R., Sakakura, T. Epithelial induction of stromal tenascin in the mouse mammary gland: from embryogenesis to carcinogenesis. *Dev Biol* 128, 245-255 (1988)

43. Zagzag, D., Friedlander, D.R., Miller, D.C., Dosik, J., Cangiarella, J., Kostianovsky, M., Cohen, H., Grumet, M., Greco, M.A. Tenascin expression in astrocytomas correlates with angiogenesis. *Cancer Res* 55, 907-914 (1995)

44. Phillips, G.R., Krushel, L.A., Crossin, K.L. Domains of tenascin involved in glioma migration. *J Cell Sci* 111, 1095-1104 (1998)

45. Kurpad, S.N., Zhao, X.G., Wikstrand, C.J., Batra, S.K., McLendon, R.E., Bigner, D.D. Tumor antigens in astrocytic gliomas. *Glia* 15, 244-256 (1995)

46. Ventimiglia, J.B., Wikstrand, C.J., Ostrowski, L.E., Bourdon, M.A., Lightner, V.A., Bigner, D.D. Tenascin expression in human glioma cell lines and normal tissues. *J Neuroimmunol* 36, 41-55 (1992)

47. Treasurywala, S. and Berens, M.E. Migration arrest in glioma cells is dependent on the alphav integrin subunit. *Proc Annu Meet Am Assoc Cancer Res* 38, A1948(1997)

48. Berens, M.E., Treasurywala, S., Giese, A. Biphasic adhesion and migration responses (stimulation and suppression) to tenascin by human glioma cells. *Proc Annu Meet Am Assoc Cancer Res* 37, A482(1996)

49. Higuchi, M., Arita, N., Ohnishi, T., Hiraga, S., Kusakabe, M., Sakakura, T., Hayakawa, T. Leptomeningeal dissemination of malignant glioma: immunohistochemical analysis of tenascin and glial fibrillary acidic protein. *Clin Neuropathol* 14, 223-227 (1995)

50. Higuchi, M., Ohnishi, T., Arita, N., Hiraga, S., Hayakawa, T. Expression of tenascin in human gliomas: its relation to histological malignancy, tumor dedifferentiation and angiogenesis. *Acta Neuropathol* 85, 481-487 (1993)

51. Jallo, G.I., Friedlander, D.R., Kelly, P.J., Wisoff, J.H., Grumet, M., Zagzag, D. Tenascin-C expression in the cyst wall and fluid of human brain tumors correlates with angiogenesis. *Neurosurg* 41, 1052-1059 (1997)

52. Felding-Habermann, B. and Cheresh, D.A. Vitronectin and its receptors. *Curr Opin Cell Biol* 5, 864-868 (1993)

53. Neugebauer, K.M., Emmett, C.J., Venstrom, K.A., Reichardt, L.F. Vitronectin and thrombospondin promote retinal neurite outgrowth: developmental regulation and role of integrins. *Neuron* 6, 345-358 (1991)

54. Seiffert, D., Keeton, M., Eguchi, Y., Sawdey, M., Loskutoff, D.J. Detection of vitronectin mRNA in tissues and cells of the mouse. *Proc Natl Acad Sci USA* 88, 9402-9406 (1991)

55. Gladson, C.L. and Cheresh, D.A. Glioblastoma expression of vitronectin and the alphaVbeta3 integrin: adhesion mechanism for transformed glial cells. *J Clin Invest* 88, 1924-1932 (1991)

56. Gladson, C.L., Wilcox, J.N., Sanders, L., Gillespie, G.Y., Cheresh, D.A. Cerebral microenvironment influences expression of the vitronectin gene in astrocytic tumors. *J Cell Sci* 108, 947-956 (1995)

57. Berens, M.E., Rief, M.D., Loo, M.A., Giese, A. The role of extracellular matrix in human astrocytoma migration and proliferation studied in a microliter scale assay. *Clin Exp Metast* 12, 405-415 (1994)

58. Gladson, C.L. and Pijuan-Thompson, V. PDGF stimulates vitronectin-directed migration and causes a localization of integrin alphavbeta3 and alphavbeta5 focal adhesions to the cell cortex in malignant astrocytoma cells. *Proc Annu Meet Am Assoc Cancer Res* 38, A953(1997)

59. Kost, C., Stuber, W., Ehrlichy, H.J., Pannekoek, H., Preissner, K.T. Mapping of binding sites for heparin, plasminogen activator inhibitor-1, and plasminogen to vitronectin's heparin-binding region reveals a nevel vitronectin-dependent feedback mechanism for the control of plasmin formation. *J Biol Chem* 267, 12098-12105 (1992)

60. Chambers, S.K., Ivins, C.M., Carcangiu, M.L. Plasminogen activator inhibitor-1 is an independent poor prognostic factor for survival in advanced stage epithelial ovarian cancer patients. *Int J Cancer* 79, 449-454 (1998)

61. Knoop, A., Andreasen, P.A., Andersen, J.A., Hansen, S., Laenkholm, A.V., Simonsen, A.C., Andersen, J., Overgaard, J. Prognostic significance of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 in primary breast cancer. *Br J Cancer* 77, 932-940 (1998)

62. Grndahl-Hansen, J., Hilsenbeck, S.G., Christensen, I.J., Clark, G.M., Osborne, C.K., Brunner, N. Prognostic significance of PAI-1 and uPA in cytosolic extracts obtained from node-positive breast cancer patients. *Breast Cancer Res Treat* 43, 153-163 (1997)

63. Hofmann, R., Lehmer, A., Hartung, R., Robrecht, C., Buresch, M., Grothe, F. Prognostic value of urokinase plasminogen activator and plasminogen activator inhibitor-1 in renal cell cancer. *J Urol* 155, 858-862 (1996)

64. Foekens, J.A., Look, M.P., Peters, H.A., van Putten, W.L., Portengen, H., Klijn, J.G. Urokinase-type plasminogen activator and its inhibitor PAI-1: predictors of poor response to tamoxifen therapy in recurrent breast cancer. *J Natl Cancer Inst* 87, 751-756 (1995)

65. Kobayashi, H., Fujishiro, S., Terao, T. Impact of urokinase-type plasminogen activator and its inhibitor type 1 on prognosis in cervical cancer of the uterus. *Cancer Res* 54, 6539-6548 (1994)

66. Nekarda, H., Schmitt, M., Ulm, K., Wenninger, A., Vogelsang, H., Becker, K., Roder, JD, Fink, U., Siewert, J.R. Prognostic impact of urokinase-type plasminogen activator and its inhibitor PAI-1 in completely resected gastric cancer. *Cancer Res* 54, 2900-2907 (1994)

67. Yamamoto, M., Sawaya, R., Mohanam, S., Rao, V.H., Bruner, J.M., Nicolson, G.L., Ohshima, K., Rao, J.S. Activities, localizations, and roles of serine proteases and their inhibitors in human brain tumor progression. *J Neurooncol* 22, 139-151 (1994)

68. Bindal, A.K., Hammoud, M., Shi, W.M., Wu, S.Z., Sawaya, R., Rao, J.S. Prognostic significance of proteolytic enzymes in human brain tumors. *J Neurooncol* 22, 101-110 (1994)

69. Rao, J.S., Rayford, A., Morantz, R.A., Festoff, B.W., Sawaya, R. Increased levels of plasminogen activator inhibitor-1 (PAI-1) in human brain tumors. *J Neurooncol* 17, 215-221 (1993)

70. Yamamoto, M., Sawaya, R., Mohanam, S., Loskutoff, D.J., Bruner, J.M., Rao, V.H., Oka, K., Tomonaga, M., Nicolson, G.L., Rao, J.S. Expression and cellular localization of messenger RNA for plasminogen activator inhibitor type 1 in human astrocytomas in vivo. *Cancer Res* 54, 3329-3332 (1994)

71. Uhm, J.H., Dooley, N.P., Villemure, J.G., Yong, V.W. Mechanisms of glioma invasion: role of matrix-metalloproteinases. *Can J Neurol Sci* 24, 3-15 (1997)

72. Rooprai, H.K. and McCormick, D. Proteases and their inhibitors in human brain tumours: a review. *Anticancer Res* 17, 4151-4162 (1997)

73. Rao, J.S., Steck, P.A., Tofilon, P., Boyd, D., Ali-Osman, F., Stetler-Stevenson, W.G., Liotta, L.A., Sawaya, R. Role of plasminogen activator and of 92-KDa type IV collagenase in glioblastoma invasion using an in vitro matrigel model. *J Neurooncol* 18, 129-138 (1993)

74. Brooks, P.C., Stromblad, S., Sanders, L.C., von Schalscha, T.L., Aimes, R.T., Stetler-Stevenson, W.G., Quigley, J.P., Cheresh, D.A. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell* 85, 683-693 (1996)

75. Giese, A., Loo, M.A., Tran, N., Haskett, D., Coons, S.W., Berens, M.E. Dichotomy of astrocytoma migration and proliferation. *Int J Cancer* 67, 275-282 (1996)

76. Stromblad, S., Becker, J.C., Yebra, M., Brooks, P.C., Cheresh, D.A. Suppression of p53 activity and $p21^{WAF/CIP1}$ expression by vascular cell integrin alphaVbeta3 during angiogenesis. *J Clin Invest* 98, 426-433 (1996)

77. Brooks, P.C., Montgomery, A.M., Rosenfeld, M., Reisfeld, R.A., Hu, T., Klier, G., Cheresh, D.A. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79, 1157-1164 (1994)

78. Brooks, P.C., Clark, R.A.F., Cheresh, D.A. Requirement of vascular integrin alphaVbeta3 for angiogenesis. *Science* 264, 569-571 (1994)

79. Engleman, V.W., Nickols, G.A., Ross, F.P., Horton, M.A., Griggs, D.W., Settle, S.L., Ruminski, P.G., Teitelbaum, S.L. A peptidomimetic antagonist of the alpha(v)beta3 integrin inhibits bone resorption in vitro and prevents osteoporosis in vivo. *J Clin Invest* 99, 2284-2292 (1997)

80. Carron, C.P., Meyer, D.M., Pegg, J.A., Engleman, V.W., Nickols, M.A., Settle, S.L., Westlin, W.F., Ruminski, P.G., Nickols, G.A. A peptidomimetic antagonist of the integrin alpha(V)beta(3) inhibits Leydig cell tumor growth and the development of hypercalcemia of malignancy. *Cancer Res* 58, 1930-1935 (1998)

81. Gladson, C.L. Expression of integrin alphaVbeta3 in small blood vessels of glioblastoma tumors. *J Neuropathol Exp Neurol* 55, 1143-1149 (1996)

82. Chen, X.M., Wang, J.Z., Fu, B., Yu, L.F. RGD-containing peptides trigger apoptosis in glomerular mesangial cells of adult cells of adult human kidneys. *Biochem Biophys Res Commun* 234, 594-599 (1997)

83. Pierschbacher, M.D. and Ruoslahti, E. Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. *J Biol Chem* 262, 17294-17298 (1987)

84. Bourdon, M.A. Characterization of a human glioma extracellular matrix antigen by monoclonal antibody: in vitro and in vivo expression, immunochemical characterization, and monclonal antibody tumor localization. *Diss Abstr Int (Sci)* 45, 2504B-5B (1985)

85. Bourdon, M.A., Coleman, R.E., Bigner, D.D. The potential of monoclonal antibodies as carriers of radiation and drugs for immunodetection and therapy of brain tumors. *Prog Exp Tumor Res* 28, 79-101 (1984)

86. Bourdon, M.A., Wikstrand, C.J., Furthmayr, H., Matthews, T.J., Bigner, D.D. Human glioma-mesenchymal extracellular matrix antigen defined by monoclonal antibody. *Cancer Res* 43, 2796-2805 (1983)

87. Zalutsky, M.R., Archer, G.E., Garg, P.K., Batra, S.K., Bigner, D.D. Chimeric anti-tenascin antibody 81C6: increased tumor localization compared with its murine parent. *Nucl Med Biol* 23, 449-458 (1996)

88. Schold, S.C., Jr., Zalutsky, M.R., Coleman, R.E., Glantz, M.J., Friedman, A.H., Jaszczak, R.J., Bigner, S.H., Bigner, D.D. Distribution and dosimetry of I-123-labeled monoclonal antibody 81C6 in patients with anaplastic glioma. *Invest Radiol* 28, 488-496 (1993)

89. Bigner, D.D., Brown, M.T., Friedman, A.H., Coleman, R.E., Akabani, G., Friedman, H.S., Thorstad, W.L., McLendon, R.E., Bigner, S.H., Zhao, X.G., Pegram, C.N., Wikstrand, C.J., Herndon, J.E., 2nd, Vick, N.A., Paleologos, N., Cokgor, I., Provenzale, J.M., Zalutsky, MR.

Iodine-131-labeled antitenascin monoclonal antibody 81C6 treatment of patients with recurrent malignant gliomas: phase I trial results. *J Clin Oncol* 16, 2202-2212 (1998)

90. Zutter, M.M., Santoro, S.A., Staatz, W.D., Tsung, Y.L. Re-expression of the alpha 2 beta 1 integrin abrogates the malignant phenotype of breast carcinoma cells. *Proc Natl Acad Sci USA* 92, 7411-7415 (1995)

91. Keely, P.J., Fong, A.M., Zutter, M.M., Santoro, SA. Alteration of collagen-dependent adhesion, motility, and morphogenesis by the expression of antisense alpha 2 integrin mRNA in mammary cells. *J Cell Sci* 108, 595-607 (1995)

92. Bartolazzi, A., Cerboni, C., Nicotra, M.R., Mottolese, M., Bigotti, A., Natali, P.G. Transformation and tumor progression are frequently associated with expression of the alpha 3/beta 1 heterodimer in solid tumors. *Int J Cancer* 58, 488-491 (1994)

93. Dedhar, S., Argraves, W.S., Suzuki, S., Ruoslahti, E., Pierschbacher, M.D. Human osteosarcoma cells resistant to detachment by an Arg-Gly- Asp-containing peptide overproduce the fibronectin receptor. *J Cell Biol* 105, 1175-1182 (1987)

94. Van Waes, C. and Carey, T.E. Overexpression of the A9 antigen/alpha 6 beta 4 integrin in head and neck cancer. *Otolaryngol Clin North Am* 25, 1117-1139 (1992)

95. Liebert, M., Wedemeyer, G., Stein, J.A., Washington, R.W., Jr., Van, Waes, C., Carey, T.E., Grossman, H.B. The monoclonal antibody BQ16 identifies the alpha 6 beta 4 integrin on bladder cancer. *Hybridoma* 12, 67-80 (1993)

96. Costantini, R.M., Falcioni, R., Battista, P., Zupi, G., Kennel, SJ, Colasante, A., Venturo, I., Curio, C.G., Sacchi, A. Integrin (alpha 6/beta 4) expression in human lung cancer as monitored by specific monoclonal antibodies. *Cancer Res* 50, 6107-6112 (1990)

97. Albelda, S.M., Mette, S.A., Elder, D.E., Stewart, R., Damjanovich, L, Herlyn, M., Buck, C.A. Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. *Cancer Res* 50, 6757-6764 (1990)

98. Danen, E.H., Jansen, K.F., Van Kraats, A.A., Cornelissen, I.M., Ruiter, D.J., Van Muijen, G.N. Alpha v-integrins in human melanoma: gain of alpha v beta 3 and loss of alpha v beta 5 are related to tumor progression in situ but not to metastatic capacity of cell lines in nude mice. *Int J Cancer* 61, 491-496 (1995)

99. Felding-Habermann, B., Mueller, B.M., Romerdahl, C.A., Cheresh, D.A. Involvement of integrin aV gene expression in human melanoma tumorigenicity. *J Clin Invest* 89, 2018-2022 (1992)

100. Sanders, L.C., Felding-Habermann, B., Mueller, B.M., Cheresh, D.A. Role of alpha V integrins and vitronectin in human melanoma cell growth. *Cold Spring Harb Symp Quant Biol* 57, 233-240 (1992)

101. Mitjans, F., Sander, D., Adan, J., Sutter, A., Martinez, J.M., Jaggle, C.S., Moyano, J.M., Kreysch, H.G., Piulats, J., Goodman, S.L. An anti-alpha v-integrin antibody that blocks integrin function inhibits the development of a human melanoma in nude mice. *J Cell Sci* 108, 2825-2838 (1995)

**Key words:** Integrins, Gliomas, Invasion, Migration, Angiogenesis, Chemoresistance

Send all correspondence to: Dr. Jasti S. Rao, Associate Professor, Department of Neurosurgery, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Box 64, Houston, Texas 77030, Tel.-713-792-3266, Fax.713-794-4950, E-mail: JRao@notes.mdacc.tmc.edu

Received 1/25/ 99 Accepted 2/1/99